

PATENTDOCKET NO.: 2026-4253US3**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s) : Cieplak, W.

Group Art Unit: 1814

Serial No. : 08/483,326

Examiner: Bugaisky, G.

Filed : June 7, 1995

For : PERTUSSIS TOXIN GENE: CLONING AND EXPRESSION

DECLARATION UNDER 37 C.F.R. §1.131COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr., am named as the inventor in the above indicated patent application, and I state as follows:

1. In a Declaration dated March 24, 1997, I stated that prior to July 1, 1988, the claimed invention was conceived and reduced to practice. In fact, the invention was conceived and reduced to practice even before September 1, 1987. The results of these first experiments showing the invention are described below.

2. The cloned gene and its expression product have the laboratory designation mutant 4-1. Mutant 4-1 possesses and exhibits the characteristics disclosed in Patent applications 07/311,612 and its continuation 07/542,149.

3. Exhibit pages 1-3 include laboratory notebook pages which demonstrate ADP-ribosyltransferase assays involving various pertussis toxin mutants, including a demonstration of substantially reduced enzyme activity associated with mutant 4-

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1. On the bottom of page 1, a brief outline of the ADP-ribosyltransferase assay is provided. The samples were incubated in the presence of the acceptor G protein transducin [$\text{adenylate-}^{32}\text{P}$]NAD⁺ for 30 minutes at 37°C. The ADP-ribosyltransferase activity was measured as the extent of transfer of ^{32}P from the radiolabeled NAD⁺ to transducin. The amount of ^{32}P incorporation into transducin was determined in two ways. First, the reaction samples were incubated with trichloroacetic acid (TCA) after the addition of bovine serum albumin to precipitate the proteins. The resultant TCA pellets were air dried after an ether wash and the amount of radioactivity in each pellet was determined by Cerenkov spectrometry to provide a quantitative estimate of ADP-ribosyltransferase activity. This assay revealed the lack of detectable transferase activity in the 4-1 mutant sample (labelled 4-1 on right side of table, labelled SAM #24 on left) compared to the other mutants and the positive control (labelled "PTX" on right side of table, labelled SAM #2 on left side). Second, the TCA precipitated proteins were solubilized in electrophoresis sample buffer and separated by sodium dodecylsulfate polyacrylamide gel electrophoresis. The gel was dried on filter paper and exposed to X-ray film. Page three is a copy of the resultant autoradiograph, showing that the reaction mixture containing the 4-1 mutant (fourth lane from the left) contained little or no detectable radiolabelled transducin (as evidenced by the lack of a band corresponding to 39 kDa) when compared to reaction mixtures containing other mutants or 6A-4, a wild type version of the S1 subunit. This assay confirmed the results of the quantitative analysis described above and demonstrates that mutant 4-1 has substantially reduced ADP-ribosyltransferase activity when compared to either pertussis toxin or other mutants.

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4. Exhibit pages 4-5 show a stained protein gel and three Western blots which demonstrate the reactivity of mutant 4-1 with a monoclonal antibody called "SATO" (also known as 1B7). The protein gel (bottom half of page 4) shows the presence of protein in all of the samples, while the Western blots demonstrate the selective recognition of the antibodies used. The blots labelled "R α PTX" represent the protein samples seen in the protein gel, as reacted with a rabbit anti-pertussis antibody, called "R α PTX". This antibody was a polyclonal antibody which reacted with both PTX (control) and the 4-1 mutant (compare right-most lane and left-most lane). Similarly, the protein samples were reacted with the SATO monoclonal antibody, as seen in the blot labelled "SATO" on the top half of page 5. In these samples, the antibody reacted with both PTX (control) and the 4-1 mutant (compare right-most lane and left-most lane). These pages provided the first data demonstrating reactivity of the 4-1 mutant with a protective monoclonal antibody.

5. The actual dates on laboratory notebook pages described in section 2-4 above have been blocked out. I state that each laboratory notebook page in section 2-4 above was dated prior to September 1, 1987.

6. The work corresponding to section 2-4 above was carried out by me or a technician working under my direction in the United States.

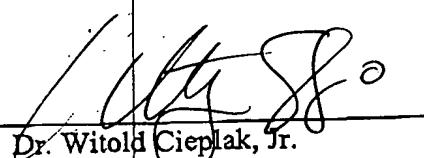
I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

DOCKET NO.: 2026-4253US3

United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 4-21-99

By:


Dr. Witold Cieplak, Jr.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/542,149

Group Art Unit: 1814

Filed: June 22, 1990

Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE:
CLONING AND EXPRESSION

DECLARATION OF WITOLD CIEPLAK, JR.

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr. hereby declare that:

(1) I have read the declaration of Dr. Jerry Keith attached hereto as Appendix 1.

(2) The copies of notebook pages attached to that declaration are copies of pages from my own notebook, as I was the one who carried out the work recorded on those pages.

(3) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

3/29/93
Date

Witold Cieplak, Jr.

12-17-91 18:16

301 402 0396

NIDR-LME

004

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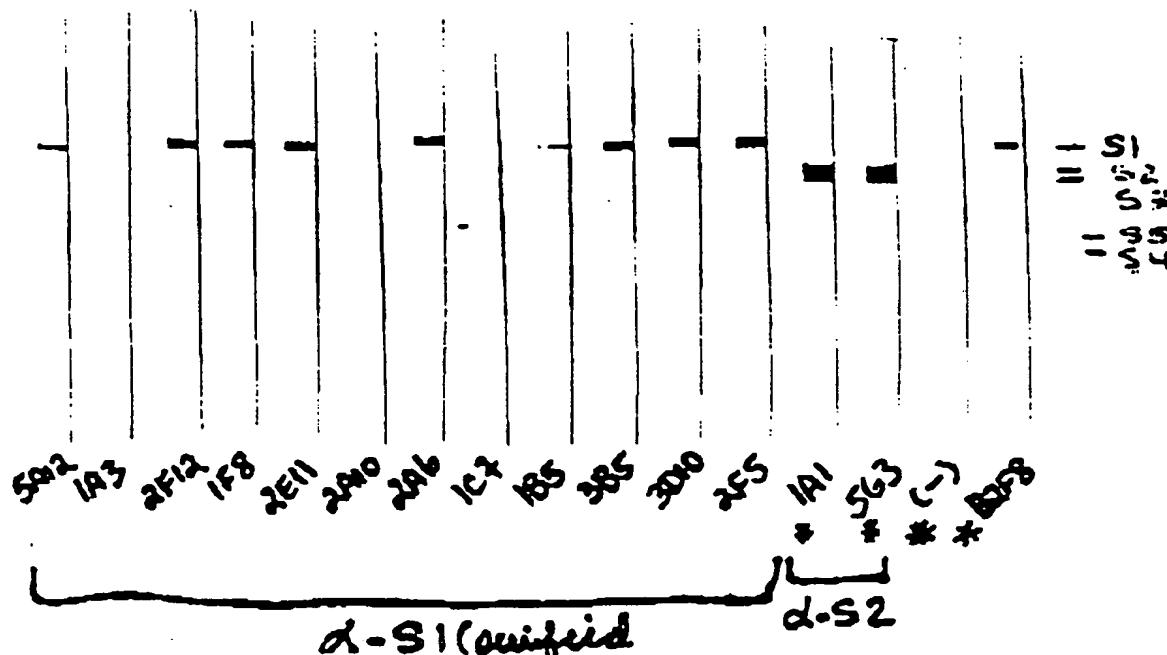
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32-

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EXHIBIT PAGE # 2

PTX
10ug



Amgen Mutant S1 protein

Immocell 1B7

----- =

EXHIBIT PAGE #3

Take samples (Analog) - dilute 1:1 w/w (see graph)

Run ~20ul for particle (5ul)

~10ul for visualizing total;

Duration

(units)

Line

1 Blanks

Blanks

2 10ul (0.5ml) Ecol 1:4

REL Stats (5ul)

3 D_{Ty} (5:1) 5ul

D_{Ty} (2.5ml)

4 6A

5 5:1

6 4:1

7 3:1 15ul

8 2:1 3ml

9 1:1

10 8:1

11 7:2

12 6:1

13 6B:2

14

15 C2:1B

Same mixture 10ul



EXHIBIT PAGE #4

Protelac gel - Drogen neutralis in unisesseli hockei
- 10 ml of 560 mg / ml in 1% Acrylamide
in 0.5 ml ea (Sent by mail).

Next time cut down on 1-1 hits for protos gal;

not in gel

PTX CSF

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

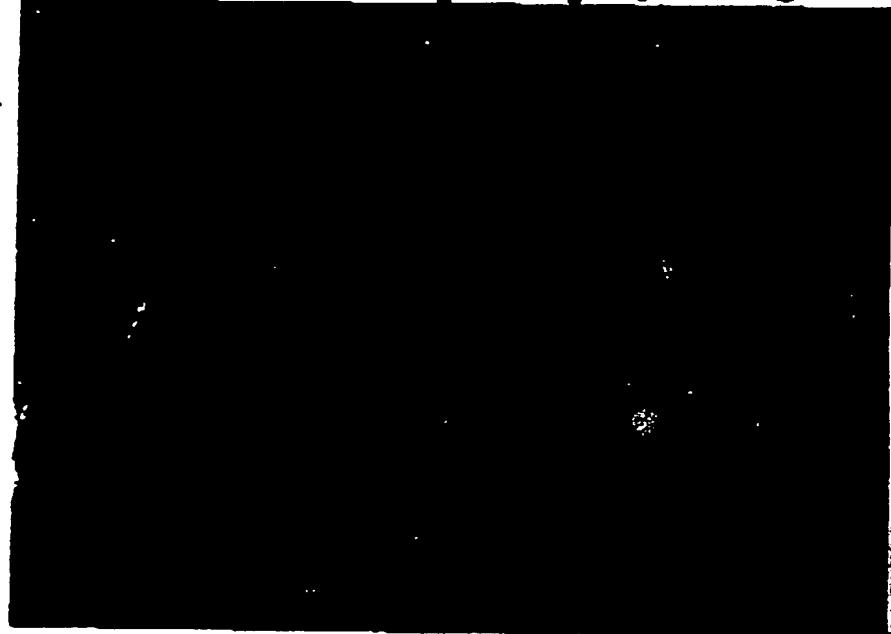


EXHIBIT PAGE #5

also

For Western B(w) (new monoclonal) and Protein

For 200ul of 1mg/ml in formamide

DIV (1ug)	Sample	2X	1X	Need
pUC18	52.2	52.2	95.5	78.4 78.4 43
S13	28.7	28.6	44.7	-remake
HSP8	33.1	33.2	132.5	too low
lys 9	39.6	39.6	120.7	
Nis 9	51.5	51.5	96.9	actually
Alu 9	58.1	58.1	83.2	0.36
Des 9	56.6	56.6	26.8	one
Nis 8:9	48.0	48.0	103.8	
Lys 58	44.5	44.5	110.9	
Cely 41	33.1	33.1	133.2	
SC441	27.2	27.2	145.5	
Des 41	33.3	33.3	133.3	

Make 200ul samples of (2) at 1mg/ml in formamide;

Load (20ul = 20ug) onto each lane of gel -

1 strip

1 blot.

(Get about .27x13 mm in moment)

Sample 2X 1X

Normal: New pS13 - 28.43 28.43 43.1

Blot 1st 2 gels = pushed w/ 2F12 and 1B7

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12-17-91 : 2:37PM :

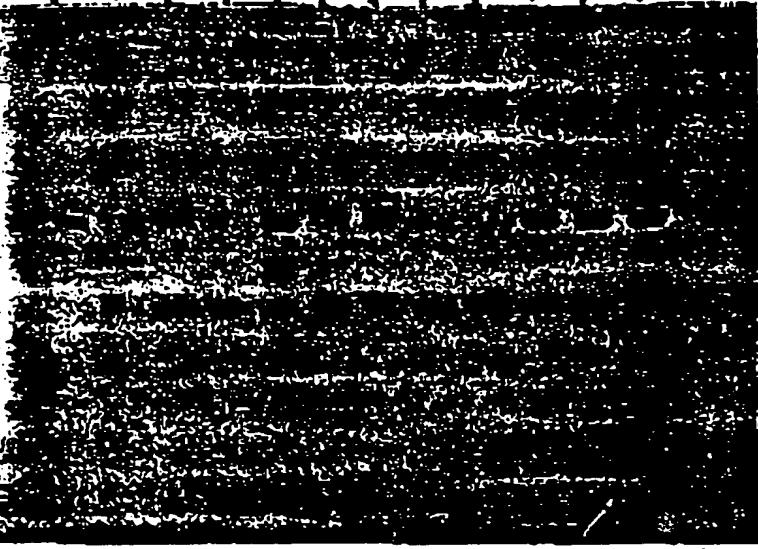
32-

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EXHIBIT PAGE #6



137

2F12<

— — — — —
— — — — —
— — — — —

EXHIBIT PAGE #7

ADP-ribosyl transferase HscyD - Arginine residue -
and S13 mutants, Hsg. mut transducin (glycan)

	Stock (S9)	For Supme	bath
Glut 51	400	12.5	987.5
6A	225	22.2	977.8
7-1	218	22.9	977.1
2-2	239	20.9	979.1
3-1	267	18.7	981.3
4-1	247	20.2	979.9
5-1	156	32.0	968.1
6-1	126	39.7	960.3
7-2	183	27.3	972.7
8-1	135	37.0	963.1
20-A	230 (total)	30.0	970.0
S11-4	199	25.1	975.0
Ala	1.34	0.373	0.127
S13	2.55	0.196	0.304
Lys 58	4.49	0.111	0.389
Asn 8.9	4.16	0.120	0.380
Asp 9	2.31	0.216	0.287
His 9	3.88	0.128	0.372
Ala 9	3.44	0.145	0.355

↓

0.5 ml/mg/ml

0.5 hr, 37°C

* Please account for new His-tagged version - no glycan;

EXHIBIT PAGE # 8

324

1	365.70	476.10	21185.40	10.00
2	442.30	536.30	22540.80	10.00
3	442.30	536.30	20702.80	10.00
4	427.20	5544.60	24332.50	10.00
5	450.30	5594.30	25768.10	10.00
6	449.30	5592.00	25200.70	10.00
7	130.70	1877.50	5925.50	10.00
8	163.50	2097.50	3630.70	10.00
9	137.90	1565.50	7625.40	10.00
10	267.60	5671.50	20435.10	10.00
11	396.20	5078.90	24578.20	10.00
12	371.60	5389.20	21945.50	10.00
13	196.10	2095.20	12523.20	10.00
14	287.20	4389.90	15388.10	10.00
15	234.30	3437.70	12737.90	10.00
16	34.20	211.90	748.00	10.00
17	32.90	187.00	754.60	10.00
18	35.00	223.20	762.40	10.00
19	400.60	4643.40	27887.60	10.00
20	379.10	4982.40	25621.90	10.00
21	446.20	6774.50	27576.10	10.00
22	38.40	228.60	896.20	10.00
23	31.40	219.80	749.50	10.00
24	31.70	178.80	649.30	10.00
25	33.30	170.70	729.40	10.00
26	32.00	178.20	745.50	10.00
27	33.10	184.70	787.40	10.00
28	31.90	165.30	696.20	10.00
29	39.00	268.20	1092.50	10.00
30	35.90	208.60	992.40	10.00
31	35.50	218.30	918.40	10.00
32	325.20	243.50	2698.70	10.00
33	35.20	195.20	809.90	10.00
34	37.70	257.30	947.30	10.00
35			348	10.00
36			963	10.00

recount

742

1120A →

108

EXHIBIT PAGE #9

Cochuet (100ng) cpm \pm SD *

upper limit

6A

 25450 ± 950

26400

1-1

 7393 ± 1367

8760

2-2

 22319 ± 3096

24415

3-1

 13549 ± 1596

90415145

4-1

 754 ± 7

761

5-1

 26361 ± 1321

27682

6-1

 764 ± 124

868

7-2

 753 ± 30

783

8-1

 926 ± 205

1131

first [20A]

 839 ± 68

907

FSI/I-4

 952 ± 9

961

* S.A. of 32P-NAO may have been a little on the low side.

114

EXHIBIT PAGE #. 10

ADP-Ribosyltransferase Activity

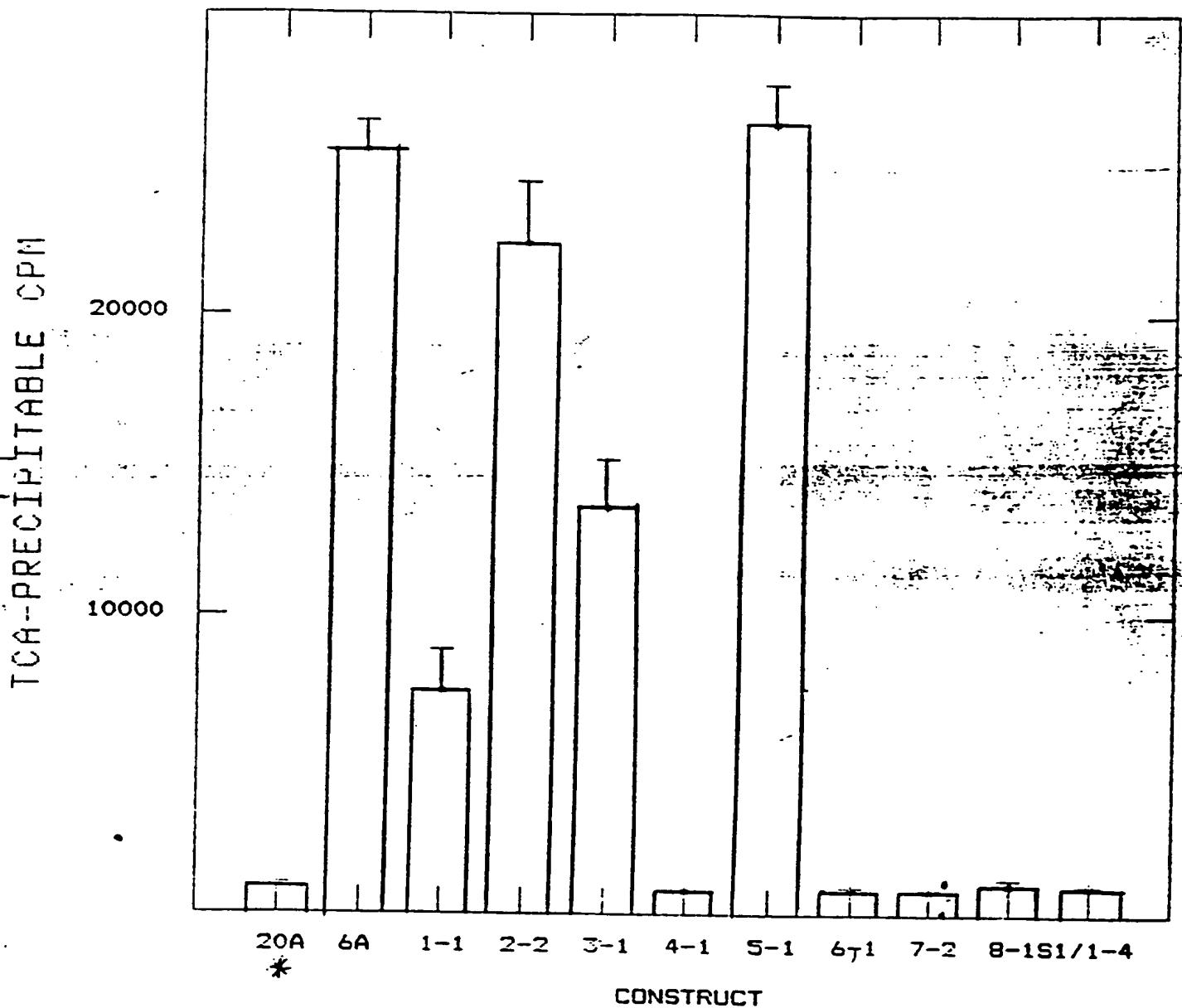


EXHIBIT PAGE #11

ADP-ribosyl transferase activity: 20A, 6A, 4-1 and S11-4

- 1) All stocks prepared in 50 mM Tris HCl, pH 8.0 protein and densitometric scans already performed
- 2) Dilutions for assay: use Tris buffer:

	①	②	③	④	⑤	⑥
6A (225 ug/ml)						
	95.6 ul	50 ul	50 ul	50 ul	450 ul	50 ul
ug/ml	4.4 ul	50 ul	50 ul	50 ul	50 ul	50 ul
	→ 10	5	2.5	1.25	0.625	0.3125
4-1 (247 ug/ml)	32.3 ul	50 ul	50 ul	50 ul		
	67.5 ul	50 ul	50 ul			
rS11-4 (199 ug/ml)	59.8 ul	50 ul	50 ul			
	40.2 ul	50 ul	50 ul			
ug/ml	80	40	20			

- 3) Use 20 ul of each prep. in 40 ul assay; assay 30' at 37°C w/ 4 ug/^T

4) Final concentrations: (ug/ml)

6A 0.185, 0.350, 0.625, 1.25, 2.5, 5.

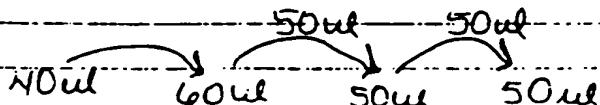
4-1 } 10, 20, 40
rS11-4 }

5) Reaction mixtures: 20 ul dilution

10 ul 4X cocktail

10 ul Transducin (400 ug/ml 50% glyco)

N.B. 20A dilute



150

1.1 Ma

END - PAGE # 12

$$4-1 \quad \frac{\% \text{ Control}}{\text{min.} = 0.51} \text{ s} = 1.625 - 0.51 \approx 1.11 \text{ min.}$$

S1/1-4 = 0.53% \rightarrow 2,000 m² decrease;

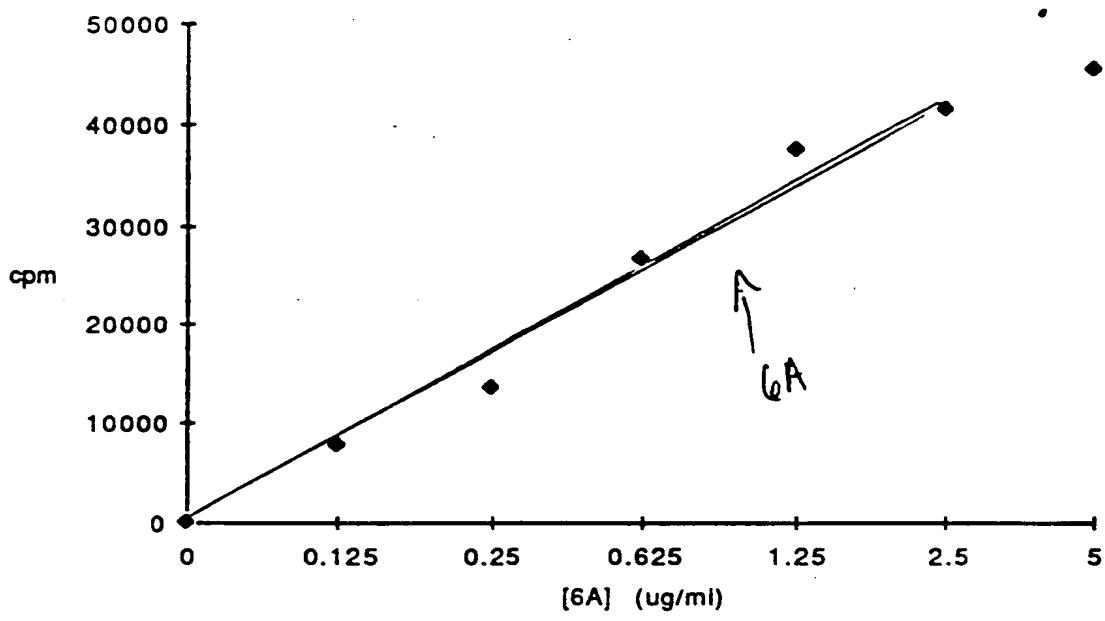
EXHIBIT PAGE # (3)

Sample	Final [ug/ml]	<u>x</u> cpm	Net cpm
Buffer	0.00	616	-
2A	0.125	8205	7589
"	0.250	14080	13464 *
"	0.625	27301	26685
"	1.25	38197	37581
"	2.5	42150	41534
"	5.0	46265	45649
20A	10.0 equiv	7688	239
"	20.0 eq	708	239
"	40 equiv	655	239
			78 cpm / ml
4-1	10	679	-39 > 5000
"	20	835	127 8481
"	40	765	110 > 10,000
S1/1-4	10	928	210 2564
"	20	1023	315 3419
"	40	9281584	929 2318

These refer to processed protein content

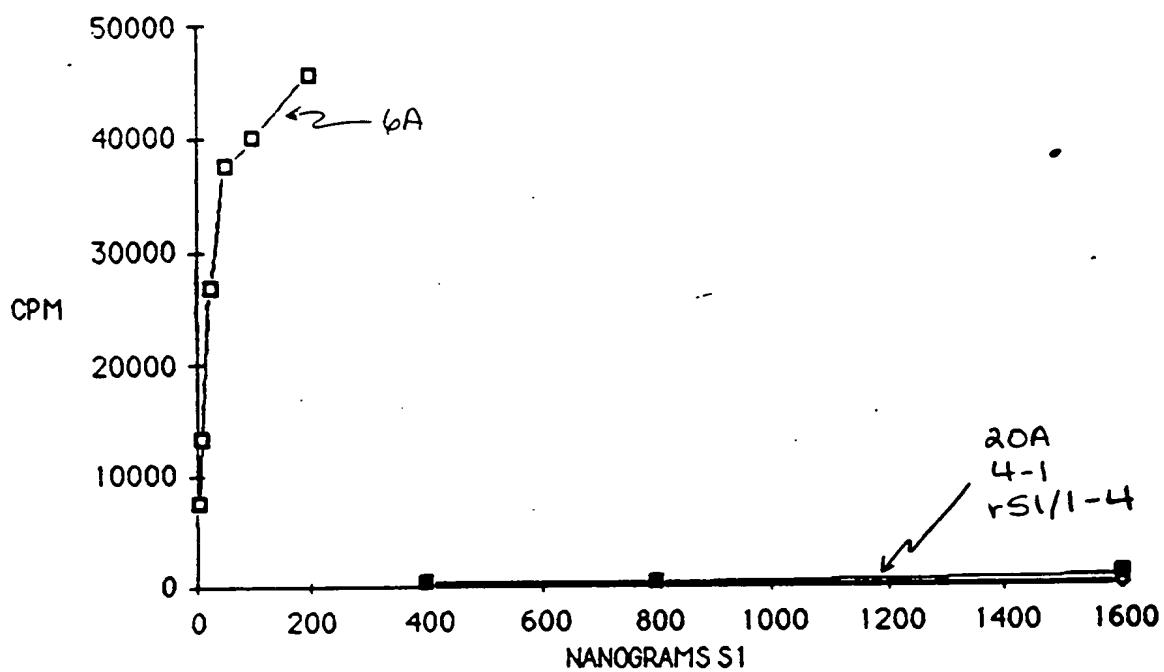
EXHIBIT PAGE # 14

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³⁵DP-Ribosylation of Tr. ... in

EXHIBIT PAGE #15



VAD glycohydrolase Assay

~~for 40 ug/ml in 100ul~~

6A	225	17.7	82.2
35A	69	57.9	42.1
39A	88	45.4	54.5
33B	113	35.3	64.7
2B	125	32.0	68.0
3B	157	25.4	74.6
1-1	75	53.3	46.6
2-2	75		
3-1	75		
4-1	75		
5-1	75		
6-1	75		
7-2	75		
8-1	75		
20A	75?		

Assayed standard fasin in duplicate; 30°C for 4 hr

50ug/ml = 1

3 pmole

$$\text{cpm} \times 1.5 \times 1.5 \div 120 \div 76.9$$

P:

EXHIBIT PAGE #11

NAD glycohydrolase - used 20A gel mol. control

all at 1ug/assay.

% Control \pm S.D. (+wh)

6A	100
35A	105.7 \pm 7.6
39A	35.3 \pm 2.3
33B	3.9 \pm 0.8
2B	1.6 \pm 1.5
3B	1.5 \pm 1.2
	-
1-1	6.1 \pm 0.98
2-2	47.6 \pm 3.1
3-1	9.1 \pm 2.0
4-1	2.2 \pm 0.4
5-1	132.1 \pm 7.4
6-1	1.7 \pm 0.4
7-2	2.2 \pm 0.6
8-1	2.6 \pm 0.4

7/1

New data

66

EXHIBIT PAGE #18

~~1ug assay~~ NAD glycohydrolase

20A (g/4μmol)	805 ± 12	Net cpm	fmols red/min/ug
6A	17,310 ± 701	16505	4.02
35A	18,257 ± 1023	17452	4.25
39A	6645 ± 304	5840	1.42
33B	1452 ± 136	647	0.15
2B	1072 ± 247	267	0.065
3B	1062 ± 184	257	0.062
20A (Int)	1558 ± 278	-	-
1-1	1814 ± 156	1009	0.24
2-2	8670 ± 399	7865	1.9
3-1	2303 ± 329	1498	0.36
4-1	1273 ± 67	372	0.09
5-1	22,615 ± 796	21,810	5.3
6-1	1685 ± 70	280	0.068
7-2	1169 ± 102	364	0.088
8-1	1233 ± 59	428	0.10

EXHIBIT 110 - #19

Need ADP-ribosyltranslase

EXHIBIT PAGE E -

NAD glycohydrolase Activity - Enzyme mutants (New preparation
Tris buffer)

21:75, 30°C., 30 μM NAD

CPM (TOTAL)

Construct	0.25 μg	0.5 μg	1.0 μg
purified SI from PTx	6,340 (1.54)	12,980.5 (0.16)	-
20A	+23	-23 (-)	
6A	1480.5 (0.36)	3,074 (0.75)	6446 (1.57)
1-1	73.5 (0.02)	254.5 (0.06)	486.5 (0.12)
2-2	562.5 (0.14)	1340 (0.33)	2734 (0.66)
3-1	125 (0.03)	419 (0.10)	882.5 (0.21)
4-1	31.5 (0.00)	-11 (0) (0)	34.5 (0.008)
5-1	1369 (0.33)	3011 (0.73)	6204 (1.51)
6-1	-5 0	-42 0	-30.5 0
7-2	-59 0	15 0	-58 0 0
8-1	-5.5 0	-4 0	204 (0.05)
SI/1-4	-60 0	-99.5 0	-64 0

$$\text{CPM} \times 1.5 \times 1.5 \times \underbrace{1.3}_{\text{sites}} \div 100 \div 120 \div \text{μg}$$

$$= .0022437 \div \text{μg} = \text{nmol/min.}$$

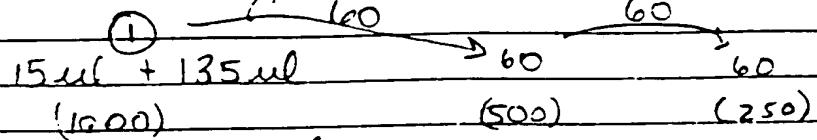
EXHIBIT PAGE 217

EXHIBIT F, GE

NAD glycohydrolase Activity Assay mixture, (cyclic)
 at 250, 500 and 1000 mg 21cmw 30°C

PSI (4100 ug/ml)

dilute to 40 ug/ml in 1:10 and 5 ml of (cyclic) pt;



Nutrients: at 75 ug/ml

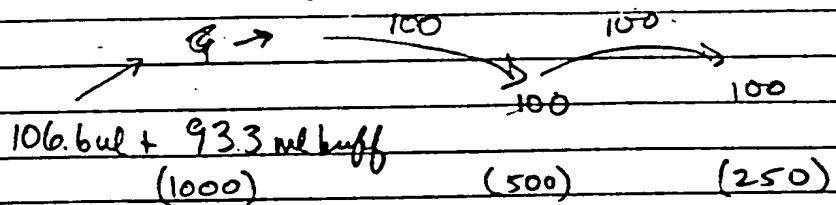


EXHIBIT THREE -

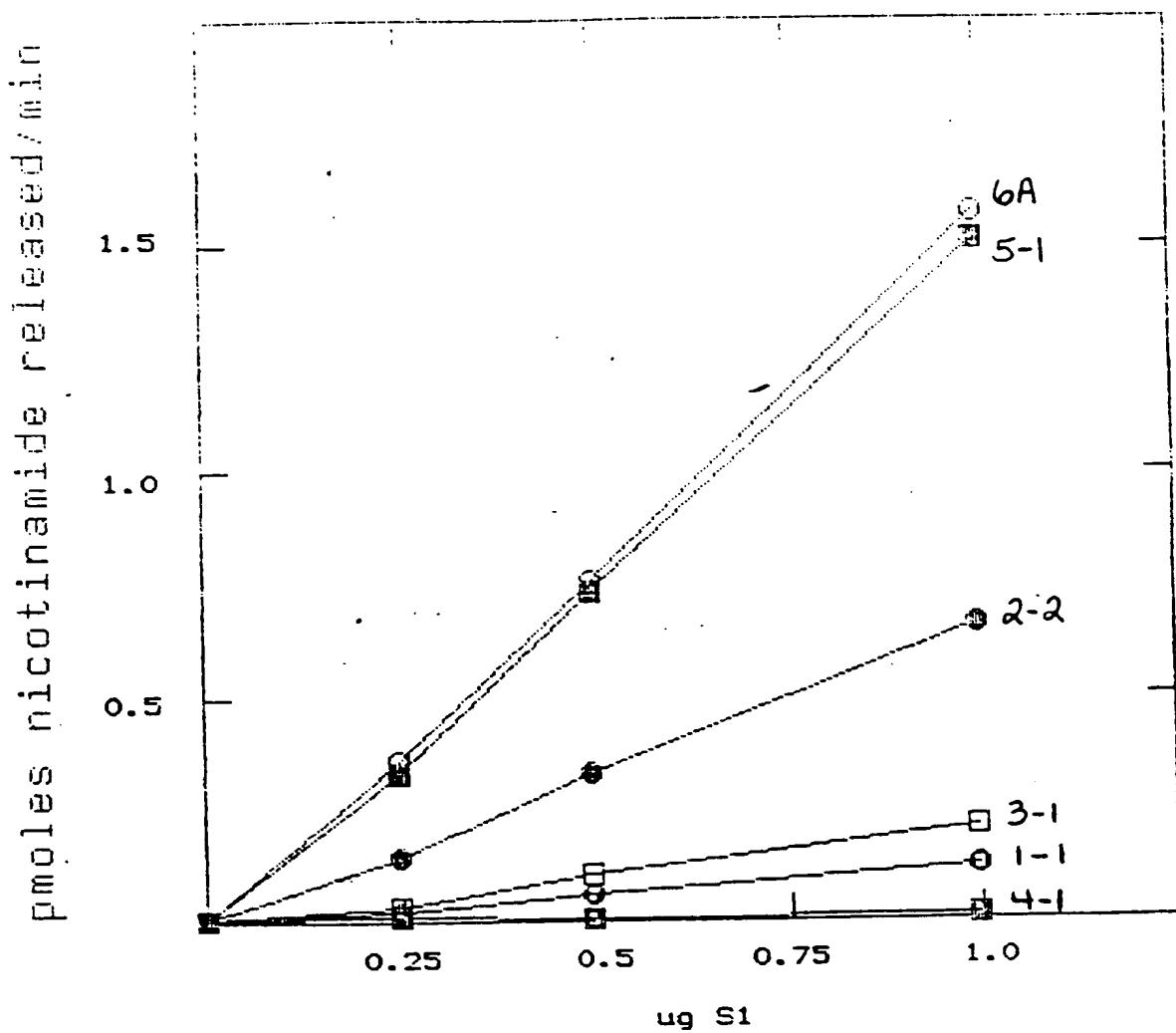
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EXHIBIT PAGE # 24

	0.25	0.5	1.0	% carbol
'A	0.36	0.75	1.57	100
1-1	0.02	0.06	0.12	7.6
2-2	0.14	0.33	0.66	42.0
3-1	0.03	0.10	0.21	13.3
4-1	0.008	0.0	0.008	0.51
5-1	0.33	0.73	1.51	96.7
6-1		0	0	0
7-2		0	0.05	3.1
8-1		0	0	0

NAD Glycohydrolase Activity

EXHIBIT PAGE #25



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket N . 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/842,149

Group Art Unit: 1814

Filed: June 22, 1990

Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE:
CLONING AND EXPRESSION

DECLARATION OF WITOLD CIEPLAK, JR.

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr. hereby declare that:

1. I previously executed a declaration for this application. In my previous declaration, I stated that I carried out the experiments recorded on notebook pages attached to a declaration by Dr. Jerry Keith. A copy of that declaration by Dr. Keith was attached to my previous declaration as Appendix 1. With the exception of the notations on the top of each page regarding exhibit page numbers, the handwriting on all of those notebook pages is my handwriting.

2. At the time I performed those experiments, it was my practice to record my notes in a looseleaf notebook. Hence, there is no notebook cover bearing my name or table of contents page reflecting those experiments.

3. During the course of my research at Rocky Mountain Laboratories, NIAID (Hamilton, Montana), I conceived that a mutation at the arginine 9 position of the amino acid sequence of the S1 subunit of *Bordetella pertussis* toxin could yield a substantially detoxified mutant comprising an epitope that contributes to

immunoprotection against *Bordetella pertussis* toxicity. I subsequently discovered that such a mutation at the arginine 9 position in fact yielded a substantially detoxified mutant comprising an epitope that contributes to immunoprotection against *Bordetella pertussis* toxicity.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

12/11/93
Date

Witold S. Cieplak
Witold Cieplak, Jr.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/542,149

Group Art Unit: 1814

Filed: June 22, 1990

Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE:
CLONING AND EXPRESSION

DECLARATION OF JERRY M. KEITH

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Jerry M. Keith, hereby declare that:

1. I have reviewed the Declaration of Dr. Cieplak attached hereto. I believe all statements in that declaration to be correct.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

December 21, 1993

Date

Jerry M. Keith

Jerry M. Keith

Serial Number: 07/542,149

-2-

Art Unit: 1814

Claims 11, 13 and 15-16 are allowable. Prosecution is now closed.

The amendment to the specification is entered, as it is clear that an inadvertent error in sequencing of the deposited parental strain occurred. The amendment does not constitute new matter.

5 The change in inventorship is permissible. It does not appear necessary to revive parent application 07/311,612 in order to grant priority (MPEP § 201.3 re continuing applications). There is, however, now no continuity between this application and 06/843,727 (Patent No. 4,883,761).

10 All claims are allowable. However, due to a potential interference, *ex parte* prosecution is SUSPENDED FOR A PERIOD OF 3 MONTHS FROM THE DATE OF THIS LETTER.

Upon expiration of the period of suspension, applicant should make an inquiry as to the status of the application.

15 Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gabriele E. Bugaisky, Ph.D. whose telephone number is (703) 308-4201.

20 Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM-1 Fax Center numbers are (703) 308-4227 and (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.



ROBERT A. WAX
SUPERVISORY PATENT EXAMINER
GROUP 180



geb

25 April 27, 1994

Current counts of TCA pellets.

PAGE

USER: 2 ID: SURVEY PRESET TIME: 21:00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR/N: RS232IN
 H#: 30 AQC/N QDC IN RGM/N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSRT
 CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSRT
 CHANNEL 3-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSRT
 DATA CALC: CTS, UNKNOWN REPLICATES: 1 NORM FACTOR: Q 1.00000
 HALF-LIFE(DAYS):N

SAM	CTS1	CTS2	CTS3	TIME
1	51.00	62.00	62.00	1.00 Buffer
2	3968.00	4089.00	4089.00	1.00 OTX(0.1ug)
3	315.00	347.00	347.00	1.00 S13 exp {8ug}
4	3560.00	3670.00	3670.00	1.00 S13 pellet (8ug)
5	721.00	494.00	494.00	1.00 S11 exp (8ug)
6	909.00	729.00	729.00	1.00 S11 pellet (8ug)
7	2857.00	2950.00	2950.00	1.00 6A-4 #2
8	3071.00	3157.00	3157.00	1.00 6A-4 #4 } 2ug
9	2354.00	2431.00	2431.00	1.00 6A-4 #6 }
10	2271.00	2361.00	2361.00	1.00 6A-4 #8 }
11	2126.00	2161.00	2161.00	1.00 Amgur rS1 (0.8ug)
12	2982.00	3086.00	3086.00	1.00 6A-3
13	3195.00	3294.00	3294.00	1.00 3CA
14	1253.00	1298.00	1298.00	1.00 33B
15	110.00	120.00	120.00	1.00 3B
16	75.00	89.00	89.00	1.00 3B
17	184.00	195.00	195.00	1.00 14B
18	87.00	97.00	97.00	1.00 14B ?
19	249.00	258.00	258.00	1.00 28B ?
20	309.00	322.00	322.00	1.00 43B ?
21	718.00	755.00	755.00	1.00 1-1
22	2087.00	2170.00	2170.00	1.00 2-2
23	731.00	818.00	818.00	1.00 3-1
24	73.00	85.00	85.00	1.00 4-1
25	3334.00	3458.00	3458.00	1.00 5-1
26	395.00	405.00	405.00	1.00 6-1 ?
27	407.00	418.00	418.00	1.00 6A-2 ?
28	372.00	377.00	377.00	1.00 7-2 ?
29	789.00	803.00	803.00	1.00 8-1 ?

} all 2ug

Assay: 10ul 4X ADPR cocktail
 20ul test sample (100ug/me - 400ug/me)
 10 ul Transducin (0.7ug)
 Stock

↓
0.5 M R 37°C

↓
1 ml 20% BSA (next use OVA + 50ul in 10ul of
 1-5 mg/ml)

50ul mixed 10% TCA

↓
V 6N. LiO₂

↓
1ml 50% TCA x 2

↓
1ml Ether
 aspirate to dryness count

ADP-ribosyl transferase

2nd gel -

Lane

1 ~~Biotin~~

2 1 Bro-Rad

3 2 1-1 1

4 3 2-2

5 4 3-1

6 5 4-1

7 6 5-1

8 7 6-1

9 8 6A-2

10 9 7-2

11 10 81

12 11 6A-4#2

13 12 6A-4#4

14 13 6A-4#6

15 14 6A-4#8

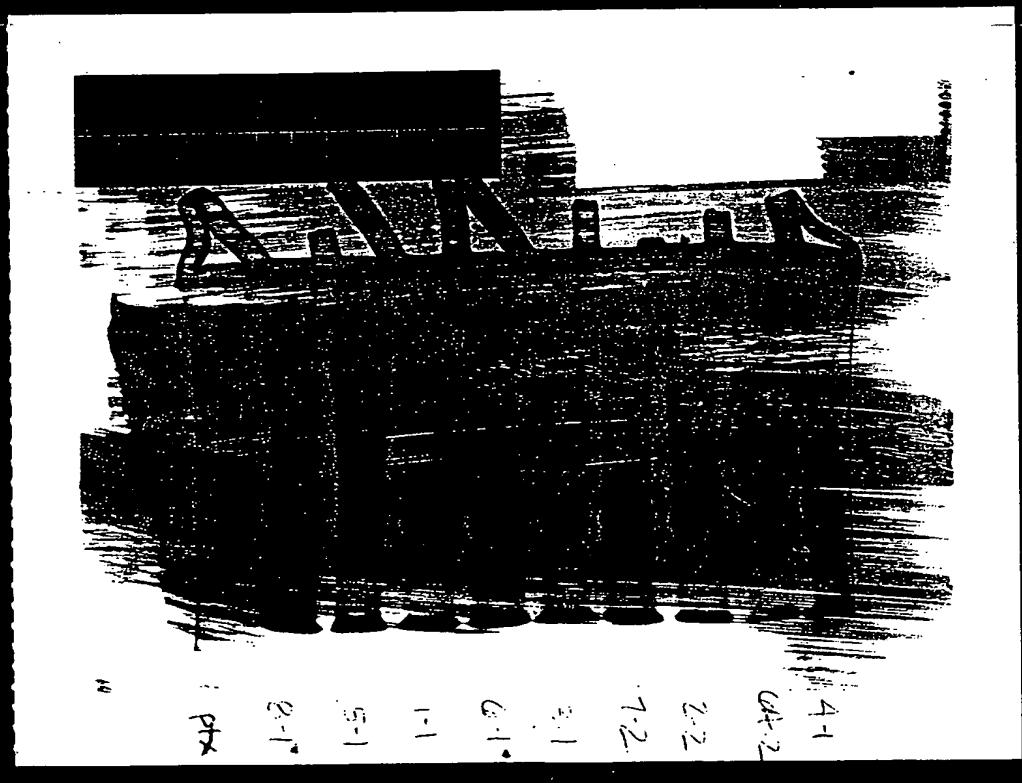
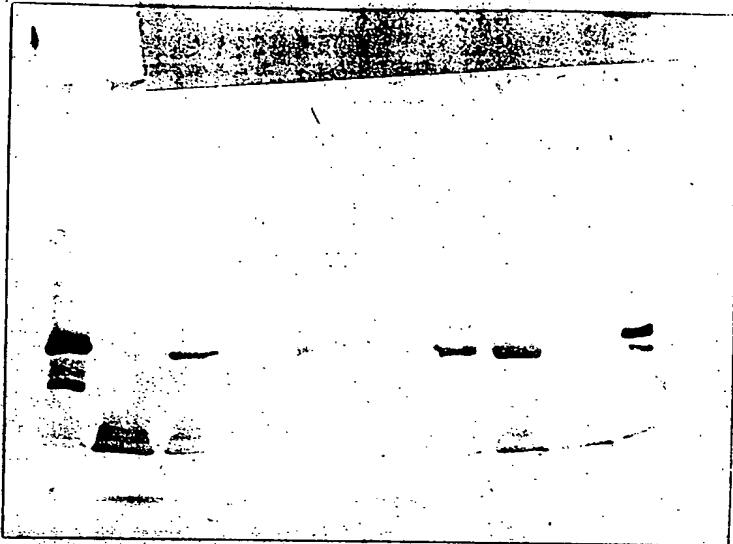
15

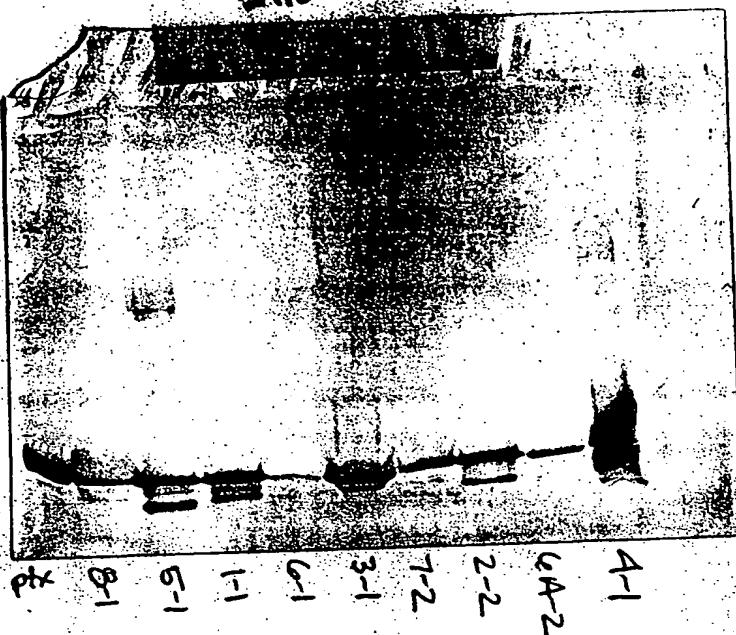
KODAK SAFETY FILM CARD

SAK →

2-2 3-1 4-1 5-1 6-1 6A-2 7-2 8-1
6A-4

R& ptx





← this is the band

Raptz

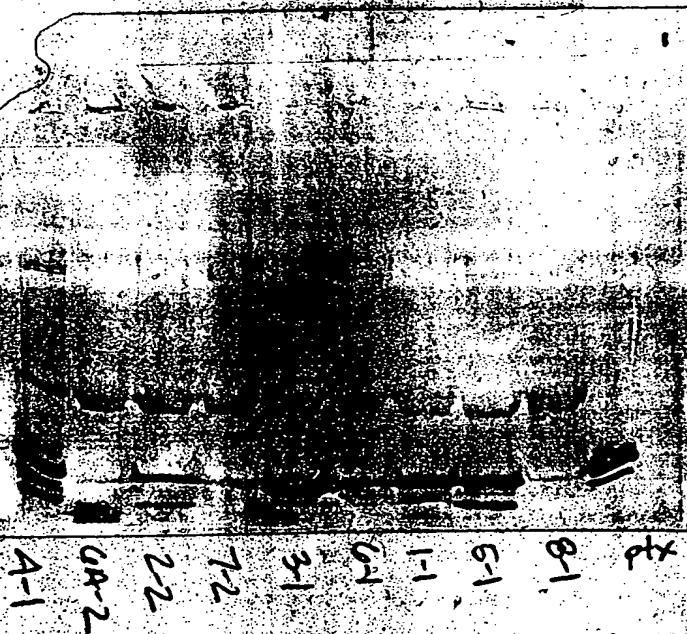


TABLE 2
Complete Nucleotide Sequence of Pertussis Toxin Gene

EcoRI
GAATTCTCCCTCGCCCTGCTTCGGGTATGGCCCCAAGGGAAACCGAACCCAAGATA
ATCGTCTGCTCAACGCCAACATCAACGAGGGCGTGCAGTCCAAGGGGGCGTCAGAGGC
TTTCCCGCCAAAGGCCAACGCCGGTATGCCACGCCGATCAGACCCGGCTTCATC
CCAGACGGAGATCCAGGGCTGGGCCGGCGTGTGCCGAAACCGGCCAACCTGAAGTAG
CAGGGCAGGCCCTCCAACGCCCATCCCCGTCGGCCGGCACCATCCGCATACGTGTTGG
CAACGCCAACGCCATGCCGTGCAGATTGCTGTACAAAACCTCGATTCTCCGTACAT
CCCCTACTGCAATCCAACACGGCATGAAACGCTCTCGGCAGAAAGTCGCGCATGCTA
CCGGTCACCGTCCGGACCGTGACGCCCTGCCATGGTGATCCGAAAAATAAGCAC
CATCAAAACGGCAGAGGGAAAGACGGGATGCCCTGCACCTGGCAATTGCCCCAAACCGCA
↑ ← M R C T R A I R Q T A
GAACAGGCTGGCTGACGTGGCTGGGATTCTGCCGTCAACGGCCCGTGACTTCGGCCG
R T G M L T V L A I L A V T A P V T S P
CATGGGCCAACGATCTCCGCCACCGTATACCGCTATGACTCCGGCCGGAGGACG
A W A D D P P A T V Y R Y D S R P P E D
TTTCCAGAACGGATTACGGCTGGGAAACAAACGACAATGTCTGACCATCTGACCG
V F Q N G F T A M G N N D H V L D H L T
GACGTTCCCTGCCAGGTGGCAGCACCAACACGGCTTCTGCCACCAAGCAGGCCG
G R S C Q V G S S N S A F V S T S S R
GCTATACCGAGGTCTATCTGAACATGCATGCCAGGAAGCCGTGGAGGCCGAAACGCCG
R Y T E V Y L E H R M Q E A V E A E R A
GCAGGGGCAACGCCACTTCATGGCTACATCTACGAAGGCCGGCAACAAATTCT
G R G T G H F I G Y I Y E V R A D N N F
ACGGGGGGCAAGCTGGTACTTCGAATAACGTGCACACTTATGGGACAAAGCCGGCGTA
Y G A A S S Y F E Y V D T Y G D N A G R
TCTCTGGGGGGCGCTGGCCACCTACCGAGCGAATATCTGGCACACCGCCGCTTCG
I L A G A L A T Y Q S E Y L A H R R I P
CCGAAACATCCGCAGGTAAACGGGTCTATCACACGCCATCACCGGGAGACCAACG
P E N I R R V T R V Y H H G I T G E T T
CCACGGAGTATTCCAACGGCTGGCTACGTCAAGCCAGCAAGACTCGGCCAAACCCCT
F T E Y S H A R Y V S Q Q T R A H P N P
ACACATGGCGAAGGTCCGTAGCGTCATCGTCGGCACATGGTGGCGATGGCCGGGTGATAG
Y T S R R S V A S I V G T L V R H A P V I

TABLE 2
Complete Nucleotide Sequence of Pertussis Toxin Gene

CCGCTTGCATGGCGGGCAAGGCCAAAGCCTGGAGGCCATGGCAGCCCTGGCTCCGAACCC
S A C H A R Q A E S S E A M A A W S E R
1300
CCGGCGAGGCCATGGTTCCTCGTGTACTACGAAAGCATGCCGTATTGGTTCTAGACCTGGC
A G E A M V L V Y Y E S I A Y S F U
[S2] →
CCAGCCCCGCCAACCTCCGGTAATTCAACAGCATGCCGAATGGACCCGAAGACGGCTCTGCC
F M P I D R K T L C
1400
ATCTCTGTCCGTTCTGCCGTGGCCCTCTGGATCTCACGTEGGCCGGCCCTCCACGG
H L L S V L P L A L L G S H V A R A R S T
1500
CAGGCATCGTCATTCCGCCGAGGAACAGATTACCCAGCATGGCAGCCCCTATGGACGCT
P G I V I P P Q E Q I T Q H G S P Y G R
GGCGGAACAAGACCCCTGCCCTGACCGTGCCGGAAATTGCCGGCCAGCCGGCATCTGCCAGG
C A N K T R A L T V A E L R G S G D L Q
1600
AGTACCTGGCTCATGTGACGCCGGCTGGTCATAATTGGCCCTTACCAATGCCACCTATC
E Y L R H V T R G W S I F A L Y D G T Y
TCGGCGGGAAATATGGCGGCGTGAATCAAGGACGGAAACACCCGGGGCCATTCGACCTGA
L G G E Y G G V I K D G T P G G A F D L
1700
AAACGACGTCTGCATCATGACCACGCCAAATACGGCTAACCCCAACGGATCACTAC
K T T F C I M T T R N T G Q P A T D H Y
1800
ACAGCAACGTACCCGCCACTGCCCTGCTCTCCAGCACCAACGGCAGGCTATGGCCGGCT
Y S H V T A T R L L S S T N S R L C A V
TCGTAGAAACGGCCAAACCCGTCAATGGCCCTGCCACCAAGCCGTATGACGGCAAGTAC
F V R S G Q P V I G A C T S P Y D G K Y
1900
GGAGCATGTACAGCCGGCTGGGAAATGCTTACCTGATCTACGTGGCCGGCATCTGCC
W S H Y S R L R K H L Y L I Y V A G I S
TACGGTCCATGTCAAGGAAGAACAGTATTACGACTATGAGGACGCCAACGTTGGAGA
V R V H V S K E E Q Y Y D Y E D A T F E
2000
CTTACGGCCCTACGGCACTCCATCTGCAATCTGGATCATCCTTATGCTGAGAACGGCT
T Y A L T G I S I C N P G S S L C U
[S3] → 2100
CCCCACTGAAACCACGGCCGGACAGGGCCGGCCCCGGCTGCCCTGCCCT
F M R A L
GGCGTGGTTGCTGGCATCCGGGGCATGACCCATCTTCCCCGGCTGGCCGACGTTCC
A W L L A S G A M T H L S P A L A D V P
2200
TTATGTGCTGGTGAAGACCAAATATGGTGGCACCCAGCGTAGCCATGAAGCCGTATGAAGT
Y V L V K T H M V V T S V A M K P Y E V

TABLE 2
Complete Nucleotide Sequence of Pertussis Toxin Gene

CACCCCGACGCCATGCTGGTCGCCGCATGCCGCCAACCTGGGGCCGCCAGCAG
T P T R M L V C G I A A K L G A A A S S
2300
CCGGACCCGCACGTGCCGTTCTGCTCGCAAGGATCTCAAGCGTCCCGGCAGCAGTCC
P D A H V P F C F G K D L K R P G S S P
2400
CATGGAAGTCATGTTGCCGCCGTCTCATGCAACAAACGGCCGCTGCCATGTTCTGGG
M E V M L R A V F M Q Q R P L R M F L G
TCCAAGCAACTCACTTTGAAGGCAAGCCCAGCTCGAACCTGATCCGGATGGTCGAATG
P K Q L T F E G K P A L E L I R M V E C
CAGCGGCAAGCAGGATTGCCCTGAAGGCACCCCATGCATACCATCGCATCCATCCTG
S G K Q D C P U FM H T I A S I L
2500
TTGTCGGCTCGGCATATAACAGCCCCGCTGACGTGCCGGCTGCCGACCCATCTGTAC
L S V L G I Y S P A D V * A G L P T H L Y
2600
AAGAACTTCACTGTCAGGAGCTGGCCTGAAACTGAAGGGCAAGAACATCAGGAGTTCTGC
K N F T V Q E L A L K L K G K N Q E F C
2700
CTGACCGCCCTCATGTCGGGCAGAACGCTGGTCCGGCGTGCCCTGTCGACCCGGGACAC
L T A F M S G R S L V R A C L S D A G H
GAGCACGACACGTGGTCGACACCAGCTGGCTTGCCATATCCGTATGCCCTCAAG
E H D T W F D T M L G F A I S A Y A L K
2800
AGCCGGATCGCGCTGACGGTGGAAAGACTGCCGTATCCGGGACTCCGGGATCTGCTC
S R I A L T V E D S P Y P G T P G D L L
GAAC TGCAAGATCTGCCGCTAACGGATAATGCGAATGAACCCCTCCGGAGGTTGACG
E L Q I C P L N G Y C E U
2900
TTCCGGCAATCCGTTGAGACGATCTCCGCCCTGGTCCATTCCGGAAACCGCA
3000
CATGCTGATCAACAAGAACAGAGCTGCTTCATCACATTCTGCCATCCGTCTGCCCT
FM L I N N K K L L H H I L P I L V L A L
GCTGGGCATGCCACGGCCAGGCCAGGCTTGCGCCAGGCATCGTATCCGGCAAGGCACT
L G M R T A Q * V A P G I V I P P K A L
3100
GTTCACCCAAACAGGGCGGCCATGGACGCTGCCGAACGGAACCCGCGCTTGACCGT
F T Q Q G G A Y G R C P N G T R A L T V
GGCCGAAC TGCGCGAACGCCGAATTGCAAGACGTATTCGCCAGATAACGCCGGCTG
A E L R G N A E L Q T Y L R Q I T P G W
3200
GTCCATATACGGTCTATGACGGTACGTACCTGGCCAGGCATGGCGGATCATCAA
S I Y G L Y D G T Y L G Q A Y G G I I K
3300
GGACGCCGCCAGGCCAGGGTTCAATTATGCCAAACTTCTGCATCACGACCATATA
D A P P G A G F I Y R E T F C I T T I Y

TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

CAAGACCGGGCAACCGGGCTGCGGATCACTACTACAGCAAGGTACCGGCCACGCCCTGCT
K T G Q P A A D H Y Y S K V T A T R L L
3400
CGCCAGCACCAACAGCAGGCTGTGCGGGTATTCTCAGGGACGGGCAATCGGTATCGG
A S T N S R L C A V F V R D G Q S V I G
AGCCTGCGCCAGCCGTATGAAGGCAGGTACAGAGACATGTACGACGCCCTGCCGCC
A C A S P Y E G R Y R D M Y D A L R R L
3500
GCTGTACATGATCTATATGTCCGGCCTTGCCTGACGGTCCACGTCAAGCAAGGAAGAGCA
L Y M I Y M S G L A V R V H V S K E E Q
3600
GTATTACGACTACGAGGGACGCCACATTCCAGACCTATGCCCTCACCGGCATTCCCTCTG
Y Y D Y E D A T F Q T Y A L T G I S L C
CAACCCGGCAGCGTCGATACTGCTGAGCCGCCGGCTGGATCTGTTGCCCTGTCCATGTT
N P A A S I C U
3700
TTCCCTGACGGATAACCGGAATGAATCCCTGAAAGACTTGAGAGCATCGCTACCGGCC
TGGCCTTCATGGCAGCCGTGACCCCTGTTGTCGCCACGCCTGCCGACCTGCCAGGCC
3800
GCGGCGGGCTGCAGCGCTGTCAACCACCTCATGGCGAGCATCGTGGTCGACTGCCGG
3900
CGGTCACTGCCACGGTGACCATGCCATAATCTGGCGGGCTACAAGCTGCTGTTCCGG
CACGCCGATGTGCTGGACGTGGTGGCTGGCTGGCGGAGCTGCTGATCGGCCATC
4000
GGCCGAAATCGCTGTTATCTGCTGACCTGAATCCGGACGTATCGAACATGCGTGTAC
GCTTTCAAGGGCTGCACCCGGCCGGATGCTGATGGCGTACCCGCCACGGCAGGCC
4100
TGTGCAGCCGGCACCATCCCTGCTGGCCATCTGGTTCAGCATCCCCTTCTGGCTT
4200
GTTTCCCGTGGCATTGCTGCCGATCCGGATCATGATCCGGCGCATGACCAAGCAGTTCCG

Sau3A
CCTGATC

The deduced amino acid sequences of the individual subunits are shown in the single letter code below the nucleotide sequence. The proposed signal peptide cleavage sites are indicated by asterisks. The start of the protein coding region for each subunit is indicated by the box and arrow over the initiation codon. Putative ribosomal binding sites are underlined. The promotor-like sequence is shown in the -35 and -10 boxes. Proposed transcriptional start site is indicated by the arrow in the CAT box. Inverted repeats are indicated by the arrows in the flanking regions.

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